

## APPENDIX A

### Summary of references for role of RNA fragments in silencing (all organisms, not only plants)

Year	Citation	Summary
1991	Fire et al., Development 113:503-14	disruption of expression of myofilament proteins <i>C. elegans</i> (nematode) by fragments of <i>unc-22</i> and <i>unc-54</i> in antisense and sense orientation
1994	Cogoni et al., Antonie Van Leeuwenhoek 65:205-9	transformation of <i>Neurospora</i> (fungus) with fragments of carotenogenic albino 1 or albino 3 genes results in suppression
1996	Cogoni et al., EMBO J 15:3153-63	fragments with approximately 132 bp of sequences homologous to the transcribed region of the native gene are sufficient to produce sense suppression in <i>Neurospora</i> (fungus)
1997	Mitzaffi et al., Cell 88:845-54	sense suppression of chalcone synthase involves 43-base paired segments of the coding region and 3' UTR of the transgene transcript that are 80% complementary.
1997	Ratcliffi et al., Science 276:1558-1560	virus cross-resistance and transgene-induced gene silencing in plants involve similar RNA-based mechanisms
1998	Fire et al., Nature 391:806-11	double-stranded RNA corresponding to fragments of exons inhibits gene expression in <i>C. elegans</i> (nematode) more effectively than sense or antisense single-stranded RNA
1998	Kennedell and Carthew, Cell 95:1017-26	double-stranded RNA corresponding to fragments of genes interferes with gene expression in <i>Drosophila</i> (fruit fly) embryos
1998	Montgomery and Fire, Trends Genet 14:255-8	Review
1999	Cogoni and Macino, Curr Opin Microbiol 2:657-62	Review
1999	Hamilton and Baulcombe, Science 286:950-952	commonalities of homology-dependent gene silencing in fungi and plants
1999	Elbashir et al., Genes Dev 15:188-200	antisense and sense small RNAs ca. 25 nucleotides are present in tomatoes with cosuppression or antisense suppression
2001	Thomas et al., Plant J 25:417-25	21- and 22-nt RNA fragments generated from double-stranded RNAs are the sequence-specific mediators of RNA interference in <i>Drosophila</i> (fruit fly)
2001	Thomas et al., Plant J 29:509-519	synthetic nucleotides with 23 - 30 base matches to GFP transgene were effective in VIGS; sequences as short as 33 nt silenced endogenous phytoene desaturase
2002	Han and Grierson Plant J 29:509-513	Small antisense RNAs, about 23 nucleotides, present in transgenic tomato plants exhibiting post-transcriptional silencing of the endogenous polygalacturonase cytoplasmic siRNA silencing (virus resistance), the silencing of endogenous messenger RNAs by miRNAs, and silencing via
2004	Baulcombe, Nature 431:356-63	DNA methylation in plants all involve the cleavage of a doublestranded RNA (dsRNA) into short 21-26-nucleotide RNAs

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Year	Citation	Gene	Type	Length	Comments
1990	Smith et al., Mol Gen Genet 224:477-81	polygadacturonase	VIGS sense	651 bp	partial cDNA ( Arg208-Thr424) from tomato, worked in <i>N. benthamiana</i>
1995	Kumagai et al., Proc Natl Acad Sci U S A 92:1679-83	phytene desaturase	VIGS sense	651 bp	partial cDNA ( Arg208-Thr424) from tomato, worked in <i>N. benthamiana</i>
1996	Hamada et al., Transgenic Res. 5:115-21	omega-3 fatty acid desaturase	VIGS antisense	651 bp	partial cDNA ( Arg208-Thr424) from tomato, worked in <i>N. benthamiana</i>
1997	Song et al., Plant Physiology 114:927-935	Peptide Transport Gene AtPTR2-B	antisense	0.5 kb	3'-flanking region and a part of the coding region
	Fan et al., Plant Cell 9:2183-2196	phospholipase D alpha	antisense	651 bp	SacI-XbaI fragment of AtPTX2-B, has 100-bp deletion of the coding
1998	Ruiz et al., Plant Cell 10:937-946	phytene desaturase	antisense	0.5 kb	region and lacks 230 bp from 3' noncoding region of cDNA
1998	El Euch et al., Plant Mol Biol 38:467-79	chalcone synthase	sense	786	DNA fragment nucleotides 1446 to 2231 of cDNA
1998	Brugiere et al., Plant Cell 11:1995-2012	cytosolic glutamine synthetase	antisense	415 nt	
1999	D'Aoust et al., Plant Cell 11:2407-2418	sucrose synthase	antisense	377 nt	distinct region from 415 nt fragment
1999	Thomas et al., Plant J 25:417-25	gfp transgene	antisense	212 nt	from within 415 nt fragment
2001		phytene desaturase	antisense	400 bp	
2002	Stoutedijk et al., 2002 Plant Physiol	delta-12-desaturase	antisense	274 bp	
			antisense	538 bp	
			sense	23 - 30 nt	
			antisense	23 - 30 nt	
			sense	33, 51, 52 nt	
			antisense	33, 51, 368 nt	
			sense	120 bp	
			antisense	480 bp	
			sense	1103 bp	
2003	Laurie et al., J Exp Bot 54:739-747	SNF1-related protein kinase-1	antisense	527 bp	
2003	Liu et al., Transgenic Res 12:71-82	Waxy gene	antisense	756 bp	
2004	Popescu and Turner, Plant J 39:29-44	ribosomal protein L3	inverted repeat	326 bp	
2004	Stephurin et al., PLoS Biol 2:E217	putrescine N-methyl transferase	inverted repeat	0.9 kb	
2005	Nunes-Nesi et al., Plant Physiol 137:611-622	mitochondrial malate dehydrogenase	antisense	996 bp	
2006	Gavilano et al., J Agric Food Chem 54:9071-9078	Nicotine N-demethylase	inverted repeat	99 bp	same construct as in Siminszky et al. 2005
2006	Cao et al., Plant Cell Rep 24:715-23	cytochrome P450 CYP46MF	antisense	298bp	
2006	Lou and Baldwin, Plant Physiol 140:126-36	germin-like protein	antisense	256 bp	
2006	Diretto et al., BMC Plant Biol 6:13	lycopene epsilon cyclase	VIGS	256 bp	
2006	Yang et al., Plant Mol Biol 62:85-95	chloroplast HSP10/CipB	antisense	0.75 Kb	
2007	Gibon et al., Plant J 50:993-1005	lumarate hydratase	antisense		
2007	Potocny et al., New Phytol	pollen specific NADPH oxidase	oligonucleotides	18 b	direct introduction of synthetic oligonucleotides